Title

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Journal

American Journal of Respiratory Cell and Molecular Biology, 51(6)

ISSN

1044-1549

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Publication Date

2014-12-01

DOI

10.1165/rcmb.2014-0021tr

Peer reviewed

The Crossroads of Iron with Hypoxia and Cellular Metabolism
Implications in the Pathobiology of Pulmonary Hypertension

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Abstract

The pathologic hallmark of pulmonary arterial hypertension (PAH) is pulmonary vascular remodeling, characterized by endothelial cell proliferation, smooth muscle hypertrophy, and perivascular inflammation, ultimately contributing to increased pulmonary arterial pressures. Several recent studies have observed that iron deficiency in patients with various forms of PAH is associated with worsened clinical outcome. Iron plays a key role in many cellular processes regulating the response to hypoxia, oxidative stress, cellular proliferation, and cell metabolism. Given the potential importance of iron supplementation in patients with the disease and the broad cellular functions of iron, we review its role in processes that pertain to PAH.

Clinical Relevance

This manuscript details the complexities of how disordered iron metabolism, with iron deficiency being one of the most common nutritional deficits in the world, may act to alter the pathobiology of pulmonary vascular disease and other hypoxia-related illnesses.

Pulmonary arterial hypertension (PAH), strictly defined by a pulmonary arterial pressure ⩾ 25 mm Hg on right heart catheterization (1), is a heterogeneous disorder. A host of etiologies, including heritable, idiopathic (i.e., idiopathic PAH [IPAH]), infectious (HIV, schistosomiasis), connective tissue disease, and hypoxic conditions (pulmonary obstructive diseases, interstitial lung diseases) have been identified as causing the characteristic pathologic vascular changes (2). These contribute to a progressive increase in pulmonary vascular resistance due to precapillary pulmonary microangiopathy (3) and eventual right ventricular failure (4). Although primary PAH is classically associated with a grave prognosis (5, 6), modern therapies have resulted in improved exercise performance, quality of life, and, in some instances, reduced mortality (7). However, despite with the use of these new pharmacologic agents, the pulmonary vascular lesions and considerable morbidity and mortality persist (8).

Recently, it has been clinically observed that iron deficiency has an increased prevalence in a population of patients with IPAH as measured by reduced serum iron, elevated circulating transferrin levels, and decreased transferrin saturation. It has been found that iron deficiency is associated with increased morbidity and mortality in IPAH and scleroderma-related PAH (9–12). Furthermore, it has been suggested that iron infusions may attenuate hypoxic vasoconstriction (13, 14), and trials are underway to evaluate the effect of intravenous iron infusions in patients with primary PAH (15). These initiatives are being performed despite a paucity of mechanistic evidence concerning the pathobiologic relationship between pulmonary hypertension (PH) and iron deficiency. Many of the core pathobiologic features of PH, such as plexogenic arteriopathy, smooth muscle vasoconstriction, and inflammation (16), involve pathways that may depend on iron, and it is clear that iron homeostasis is entwined with cellular responses to hypoxia, cellular proliferation, and mitochondrial function. In contrast to iron deficiency, several hemoglobinopathies have been associated with increased prevalence of PH, which has been identified as a prognostic indicator in these disorders, highlighting the complex and multifactorial nature by which iron influences pulmonary vascular disease. The increased understanding of the role of iron in modifying these key disease pathways may lead to novel insights into the pathobiology of PH. This effort may provide

(Received in original form January 17, 2014; accepted in final form June 27, 2014)

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Am J Respir Cell Mol Biol Vol 51, Iss 6, pp 721–729, Dec 2014

Originally Published in Press as DOI: 10.1165/rcmb.2014-0021TR on July 2, 2014
Internet address: www.atsjournals.org

new therapeutic insights because current therapies are focused on pulmonary vasodilation rather than on reversing pulmonary vascular or right ventricular pathologic remodeling.

Here, we review iron metabolism and homeostasis and then focus on several pathobiologic links to PH, notably, how dysregulated iron metabolism may alter the hypoxic response of the pulmonary vasculature, oxidative stress, and cellular metabolism.

PH and Iron Deficiency: Prevalence and Prognosis

It has been demonstrated that iron deficiency is highly prevalent in IPAH and hereditary PAH, with rates ranging from 30.1 to 63%, which is much higher than the general population (10). Another recent study reported that 41% of patients with systemic sclerosis–related PH are iron deficient and that patients with systemic sclerosis without PH demonstrated only a 16% prevalence of iron deficiency (11). In these observational studies, patients did not demonstrate overt anemia compared with control patients. When patients with chronic thromboembolic disease were analyzed, the rates of iron deficiency were only 4.9%, suggesting that iron deficiency is unique to group 1 PAH (9).

Retrospective analysis has demonstrated that overt anemia correlates with increased risk of death in PH. In one study, after adjustment for known predictors of death and PH etiology, anemic patients were 3.3 times more likely to die than nonanemic patients (17). In a separate observational study, it was found that of 70 patients with IPAH, 43% were iron deficient and had significantly lower 6-minute walk distance (6MWD) than iron-replete patients, but hemodynamic indices were not significantly different. Furthermore, when the iron-deficient individuals were split into anemic and nonanemic groups, there was no significant difference in 6MWD (12). This suggests that poor functional status and outcomes may be related more to defects triggered by low systemic iron than to simple anemia. Supporting this is the fact that red blood cell distribution width, which is elevated in iron deficiency, has greater utility than established biomarkers—including N-terminal fragment of the prohormone B-type natriuretic peptide, growth differentiation factor-15, IL-6, and serum creatinine—in predicting mortality among patients with PH (18, 19). Offering further evidence that iron status modulates the disease process of PH, zinc-protoporphyrin, a highly sensitive marker of iron metabolic abnormalities and absolute deficiency, has been demonstrated to be significantly increased in individuals with IPAH and in sleep apnea–associated PH groups relative to control subjects, patients with asthma, and those with secondary PAH. Moreover, elevations of zinc-protoporphyrin strongly correlated with disease severity as determined by echocardiographic pulmonary arterial systolic pressures (PASP) and 6MWD measurements (20).

These observations highlight the preponderance of iron deficiency anemia in several forms of group 1 PAH, the mechanisms of which remain unclear. There are multiple studies demonstrating that the presence of iron deficiency correlates with worsened functional status and mortality. These data suggest an important link between iron deficiency and the pathobiology of PH and highlight the need for retrospective and mechanistic studies of this relationship, which may lead to novel insights and therapeutic strategies.

Effects of Iron Supplementation on PH

It has been observed that iron replacement may be hampered in patients with PH. In one study, patients with IPAH and iron deficiency were given oral iron preparations. At the end of 4 weeks, only 11% of the patients who had completed the intervention demonstrated significant increases in their serum iron or ferritin levels, suggesting underlying absorptive or release dysfunction in PH (12). Nevertheless, iron—or chelation in converse (13)—appears to modulate the degree of PH. As outlined below, iron participates in the control of hypoxic transcriptional controls, including stabilization of hypoxia-inducible factors, thereby affecting pulmonary vascular responses.

Alveolar hypoxia triggers demonstrable elevation in pulmonary pressures that is attributable to hypoxic vasoconstriction and pulmonary vascular remodeling. The role of iron supplementation in hypoxic vasoconstriction has been studied in a model of acute mountain sickness (14). Briefly, acute hypoxic PH was induced in human subjects by moving them from sea level (estimated O_2 partial pressures: atmospheric, 21%; lung, 15%) to an altitude of 4,340 m (estimated O_2 partial pressures: atmospheric, 13%; lung, 7%), with a predictable and significant increase in their PASP as measured by echocardiography. After 3 days, subjects were randomized to receive intravenous infusions of iron sucrose or placebo. There was a significant (and rapid) decrease in PASP in the iron-supplemented group. In a separate trial, individuals who chronically lived at an altitude of greater than 2,500 m above sea level (O_2 partial pressures: atmospheric, 16%; lung, 10%) were identified as having chronic mountain sickness (defined by excessive erythrocytosis, hypoxemia, and the absence of chronic pulmonary disease), with baseline echocardiography demonstrating elevated PASP. Patients were then randomized to staged venesection with subsequent intravenous iron or placebo infusion. After 24 days, each group underwent crossover to receive the alternative treatment. With initial venesection, progressive development of iron deficiency correlated with worsening of pulmonary arterial pressures determined by echocardiography. This study suggests that there is a causal relationship between iron deficiency and acute hypoxic PH.

Despite the potential protective role of infused iron in pulmonary vasoconstriction in the acute setting, several clinical and pathologic characteristics underscore the differences between hypoxic and class I PAH, with hypoxia-related PH demonstrating (generally) less severe elevations in right ventricular systolic pressure, a lack of response to PAH-specific therapies, and vascular lesions characterized by medial hypertrophy of small muscular arterials and more distal arteriolar neomuscularization rather than the plexiform lesions that are characteristic in PAH (21, 22). However, recent mechanistic studies have revealed elements of hypoxia-like signaling in PAH (23, 24), warranting investigation into the effects of iron in hypoxic and nonhypoxic signaling and hypertensive pulmonary vascular remodeling.

Mechanisms of Iron Homeostasis and Links to PH

Iron is involved in many integral cellular functions in aerobic organisms, including

growth, differentiation, and metabolism. Approximately 60 to 70% of iron is used by red blood cell hemoglobin, 10% is localized in myocytes in the form of myoglobin, and the remainder is an indispensable constituent of over 1,000 different iron-dependent proteins in eukaryotic organisms (25, 26). Broadly, iron is intimately involved in oxygen transport, electron transport, and DNA synthesis, exemplified by its function as an essential cofactor of ribonucleotide reductase, whereby ribonucleotides are catalytically converted to deoxyribonucleotides (27). It is essential for functioning cytochromes, lipoxygenases, fatty acid desaturases, superoxide dismutase, mitochondrial NADH dehydrogenase (complex 1), and a number of other enzymes. Conversely, when in excess, free iron catalyzes the formation of reactive species through the Fenton reaction, damaging macrostructures within the cell (28, 29). Given its fundamental and dual nature in health and disease, a rich homeostatic system has evolved to regulate iron transport and metabolism.

The average adult male body contains roughly 4 g of iron, which is efficiently recycled within the body, with < 2 mg daily replenishment required via dietary intake. No physiologic control of iron excretion exists, leaving regulation of absorption from dietary sources the primary modulator of total body iron. Dietary iron is primarily in the form of insoluble oxidized Fe^{3+} (ferric) and must be reduced to the Fe^{2+} (ferrous) form by the apical duodenal cytochrome B before being shuttled across the intestinal epithelium by the divalent metal transporter 1 (DMT1) (30). DMT1 is an evolutionarily highly conserved protein of the natural resistance–associated macrophage protein class, consisting of transmembrane transporters that are broadly involved in divalent cation uptake (31). Once through the apical duodenal epithelium, iron is exported into the circulation by ferroportin, which works with the ferroxidase hephaestin to generate ferric iron that binds to serum transferrin, an abundant plasma glycoprotein with high affinity for ferric iron that binds iron, attenuating its reactivity and transporting it to cells. After localization at the cell wall, the transferrin/iron complex binds to transferrin receptors localized on the cell surface, with subsequent endocytosis via clathrin-coated pits. Within the endosome, acidification via proton pumps on the endosomal surface leads to protein conformational changes that result in the release of iron from transferrin and transport to the cytosol by DMT1 (32). At this point, iron functions within a wide array of cellular functions as part of the labile iron pool (33).

Hepcidin Regulation of Iron Absorption

Maintenance of systemic iron homeostasis is partially regulated by hepcidin, a defensin family member that is induced in the setting of dietary and parenteral iron loading largely in liver cells. Its central role in iron physiology is highlighted by inherited disorders of hepcidin deficiency, which result in severe forms of hemochromatosis (34). Mechanistically, hepcidin decreases absorption of iron by binding ferroportin in hepatocytes, enterocytes, and macrophages, triggering its ubiquitination and subsequent lysosomal degradation (35). Hepcidin expression is markedly up-regulated by iron overload and IL-6 driven signaling, whereas hypoxia and iron depletion lead to a decline in hepcidin levels (36, 37).

The precise mechanisms that regulate hepcidin expression remain unclear, but it is apparent that IL-6 and bone morphogenic protein receptor (BMPR) signaling are critical. IL-6, a proinflammatory cytokine that is up-regulated in PAH and is associated with higher mortality (38, 39), is sufficient to induce hepcidin expression and leads to hypoferremia in animal models of inflammation (40). However, in a study of patients with IPAH, although IL-6 levels were increased, this failed to correlate with increased hepcidin activity, arguing that IL-6 is unlikely to be the sole cause of the effect (9). BMP receptors are ubiquitously expressed receptors in the TGF-β family, which bind in a heteromeric fashion and lead to phosphorylation (thus activation) and nuclear translocation of receptor-activated SMADs, which signal through a multitude of downstream pathways, including pSmad, p38, pERK, JNK, and Akt/PI3K. Heterozygous germline mutations in BMPR type 2 (*BMPR2*) have been described in both sporadic IPAH and hereditary PAH (41). Interestingly, when *BMPR2* is disrupted by *loxP* knockout or siRNA knockdown in pulmonary arterial smooth muscle cells, there is paradoxical increased activation of SMAD and p38 by BMP6, whereas BMP2 and BMP4 resulted in reduced signaling (42). BMP6 has been demonstrated to be a major positive regulator of hepcidin expression, with BMP6 knockout mice developing severe iron overload in the setting of reduced hepcidin levels (43). It is unclear whether patients with hereditary PAH with heterozygous mutations in BMPR2 have altered BMP control of hepcidin in the liver. However, some patients with PAH have increased levels of circulating hepcidin (10), and although it remains unclear if the mechanisms above are causal, increased hepcidin may explain the difficulty in supplementing these individuals with oral iron preparations (12).

Iron Regulatory Proteins and Iron Responsive Elements

The iron regulatory proteins (IRPs) tightly regulate the flow of iron by modulating expression of target proteins posttranscriptionally by binding to cis-regulatory iron responsive elements (IREs), which are found in the untranslated regions (UTRs) of mRNA-encoding proteins involved in iron metabolism (Figure 1). In iron-replete conditions, an iron-sulfur cluster (ISC) is bound to IRP-1, which relegates it to the cytoplasm to function as an aconitase. In iron-deplete conditions, without an ISC occupying its IRE binding site, IRPs are free to bind to transcripts that contain IREs (44). In contrast, IRP-2 does not contain an ISC but is regulated through F-box and leucine-rich repeat protein 5–regulated ubiquitination and proteasomal degradation (45, 46). Although IRP-1 and IRP-2 differ in their molecular nature and regulation of expression, both bind to IREs located in the 3′ UTR of transcripts, such as transferrin receptor and likely to an alternative splice form of DMT1, resulting in transcript stabilization and facilitation of translation, leading to in increased iron uptake. In contrast, when the IRP binds to transcripts containing a 5′ UTR IRE, translation is blocked, as with transcripts of H- and L-ferritin, ferroportin, and hypoxia-inducible factor 2α (HIF2α) (33, 44, 47). From a teleological perspective, IRP repression of HIF2α via a phylogenetically conserved 5′ UTR IRE (48, 49) allows for adjustment of erythrocyte production based on iron availability because HIF2α controls erythropoietin production in the kidney.

The IRP/IRE regulatory system is physiologically essential, as indicated by

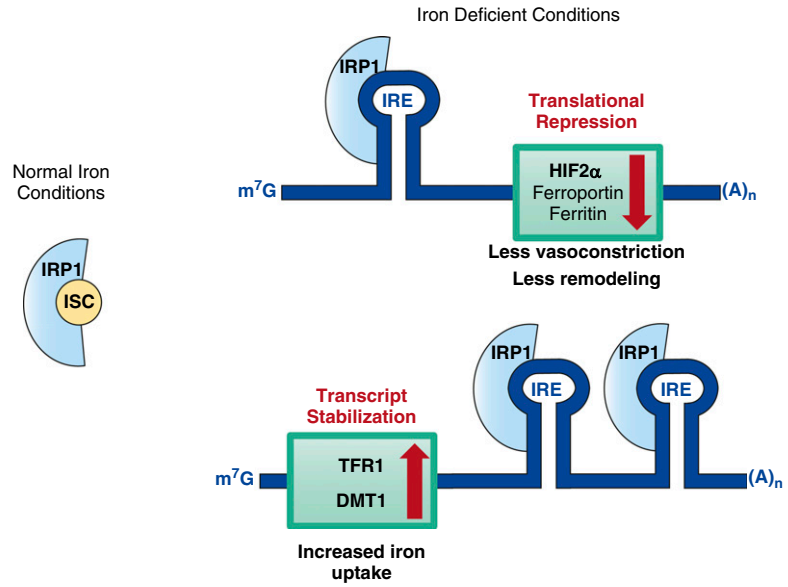

Figure 1. Iron-deplete conditions translationally repress hypoxia-inducible factor 2α (HIF2α) via iron regulatory protein (IRP) and iron responsive element (IRE) interaction. IRP1 functions as a cytosolic aconitase in iron-replete conditions when the iron sulfur cluster (ISC) is bound to the catalytic apoprotein. In iron-deplete conditions, ISCs are unavailable, which enables IRP1 to bind to either the 5′ or 3′ IREs. In the 5′ position, this results in translational repression, as is the case with HIF2α. In contrast, when IRP1 binds to a 3′ IREs (which are located on the transcripts of transferrin receptor 1 [TfR1] and divalent metal transporter 1 [DMT1]), the transcript is stabilized, resulting in increased iron uptake.

early embryonic lethality in transgenic mice that have both IRP-1 and IRP-2 knocked out. However, mice in which IRP-1 alone was knocked out did not have an immediate apparent phenotype, suggesting that IRP-1 and IRP-2 have redundant physiologic functions (i.e., IRP-2 rescues the deficiency of IRP-1) (50). However, detailed inspection found that mice with targeted deletion of IRP-1 developed polycythemia (51, 52) and spontaneous PH (53). This response was found to be due to translational derepression of HIF2α, with a subsequent increase in erythropoietin and endothelin-1 (a potent pulmonary vascular bed constrictor involved in the development of PH [54]). The PH persisted from 3 months to 1 year but without apparent hemodynamic progression. There was also the absence of muscular hypertrophy, suggesting that derepression of HIF2α may induce PH primarily via increased pulmonary vascular vasoconstriction. A known target of HIF, endothelin-1 could mediate vasoconstriction and remodeling.

IRPs act as a master transcriptional regulator modulated by iron. In addition to their well-documented role in the maintenance of iron homeostasis, IRPs are increasingly recognized to play a role in the hypoxic response. The finding that homozygous deletion of IRP-1 in mice results in spontaneous PH has led further credence to the possibility that this homeostatic axis is pertinent to the pathobiology of pulmonary vascular disease, although the exact mechanism by which IRP-1 contributes to the disease remains to be defined. Dysregulated iron metabolism, mediated by defects in the hepcidin and/or IRP regulatory axis, alters cellular availability of iron and has multiple potential effects on the pathobiology of PH.

Cellular Destinations and Functions of Iron in Relation to PH

With the vast number of enzymatically driven cellular functions that rely on iron as a cofactor, numerous iron-dependent, cellular-specific, and organ-specific processes may have a pathobiologic role in pulmonary vascular disease. Namely, there is potential interplay between iron with hypoxia and hypoxia signaling within the pulmonary vasculature, the function of iron in congestive heart failure, and iron's role in carcinogenesis.

Iron and the Hypoxic Response of the Pulmonary Vasculature

As recently reviewed, stabilization of HIF1α in hypoxic murine models results in pulmonary arterial smooth muscle cell (PASMC) proliferation, migration, and hypertrophy, ultimately contributing to the pathogenesis of PH (55). Highlighting the importance of HIFs in the development of PH, their role is not limited to World Health Organization group 3 (hypoxic) disease; increased expression of HIFs was observed in tissues of patients with non–hypoxia-related PAH (23, 56). Although similar phenotypic attenuation of PH is seen in HIF1α(+/−) and HIF2α(+/−) mice, it remains to be shown if this occurs through similar mechanisms in PASMCs (57, 58).

Targeted knock-out of IRP-1 resulted in PH via derepression of HIF2α. This suggests that, in iron-deficient conditions, there may be increased IRP-1 binding of IREs (due to decreased ISC binding to the cytosolic aconitase), leading to suppressed HIF2α translation and protection from the maladaptive PASMC remodeling. On the other hand, iron deficiency may worsen PH via HIF-dependent mechanisms. The ubiquitin ligase von Hippel-Lindau protein (VHL) mediates normoxic degradation of HIF1α and HIF2α to very low levels after prolyl hydroxylation (mediated by prolyl hydroxylase [PHD]) and subsequent proteasome-mediated degradation. PHDs require not only oxygen but also 2-oxoglutarate (α-ketoglutarate), ascorbate, and iron (Figure 2) as essential cofactors (59, 60). It has been observed that iron chelation acutely elevates pulmonary vascular resistance in normal individuals subjected to acute hypoxia, which has been attributed to increased HIF stabilization (61).

Chuvash polycythemia (CP) is a rare autosomal recessive disorder in which individuals carry a dysfunctional VHL with the missense mutation R200W, resulting in increased HIF1α and HIF2α stabilization and increased expression of downstream target genes, including erythropoietin, glucose transporter member 1, transferrin, transferrin receptor, and vascular endothelial growth factor (62). Human physiologic studies have demonstrated that patients with CP have elevated baseline

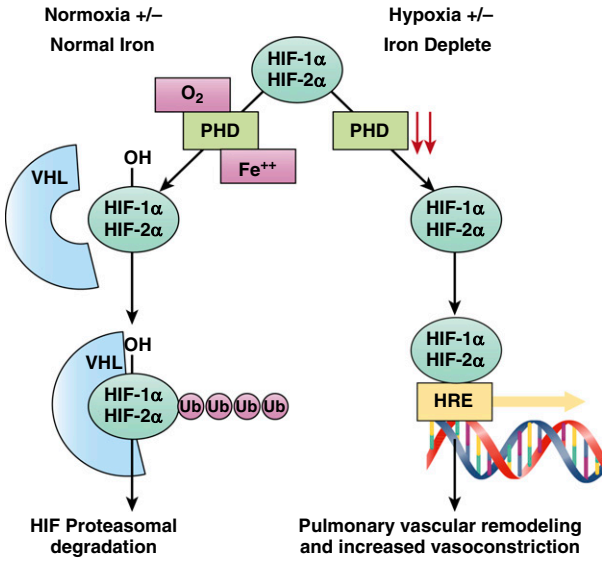

Figure 2. Iron-deplete conditions stabilize HIF via a prolyl hydroxylase (PHD)-dependent mechanism. Iron is an essential cofactor for PHD, which allows for rapid ubiquitination and degradation by Von-Hippel Lindau (VHL) protein E3 ubiquitin ligase. HRE, hormone response element.

PASP when measured by echocardiography without evidence of clinical PH. When exposed to hypoxic conditions, patients with CP exhibit extraordinary pulmonary vascular sensitivity relative to control subjects (63). It is conceivable that this is due to unchecked HIF-mediated pulmonary vasoconstriction. Furthermore, many patients with CP require phlebotomy, which results in iron deficiency (as reflected by low serum ferritin); this iron deficiency, even when corrected for reduced blood volume (as represented by left ventricular internal diastolic diameter, hemoglobin concentration, and left atrial diameter), was associated with significantly increased tricuspid regurgitation velocity, a surrogate for the severity of pulmonary vascular pressures (64).

In a murine model of CP, mice carrying the R200W mutation in the VHL gene in a homozygous fashion develop PH independent of polycythemia. HIF2α, but not HIF1α, was the driver of PH and polycythemia in the animals with CP (65). The hypertensive process is accompanied by increased perivascular inflammation and vascular remodeling. The interesting parallels and differences of the pulmonary hypertensive phenotype between IRP-1 null (as described above) and VHL R200W mutant suggest that these proximal genetic events affect the pulmonary vascular phenotype, possibly by affecting downstream effector genes. In addition to the shared up-regulation of HIF2α in both models, IRP-1 and VHL differentially targeted transcripts and proteins, respectively, may account for the specific findings in both models.

An interesting correlate is found in humans who carry HIF2α gain–of-function mutations that induce a more vasoconstrictive phenotype of PH along with an exaggerated vasoconstrictive response to hypoxia (66, 67). Two separate genome studies have demonstrated a significant increase in SNPs in *EPAS1*—the gene coding for HIF2α—in ethnic Tibetans living at elevations exceeding 4,000 m (68, 69). There is an associated decrease in hemoglobin concentration, ameliorating one of the key findings of chronic mountain sickness. It is posited that, either through this or through one of the many other transcriptional effects of HIF2α, a naturally selected survival advantage is offered by decreased HIF2α function at altitude (69).

The experimental data highlighted here demonstrate that HIF plays a role in the pathogenesis of hypoxic PH and may play a role in primary PAH. Low iron could therefore act to stabilize HIF via inhibition of 2-oxoglutarate–dependent PHDs. However, low iron, via stabilization of IRP-1, can selectively suppress HIF2α translation, leaving HIF1α to exert its prohypertensive effects. The mechanisms by which HIF levels are elevated in PAH remain unclear, but the role of iron, either through IRP-regulated repression or VHL/PHD-mediated stabilization, represents an enticing therapeutic target once mechanisms can be delineated.

Iron and Oxidative Stress

Iron's essential role in cellular biology is largely dependent on its ability to undergo cyclic oxidation and reduction. The most basic form of the Fenton reaction involves an electron transfer from hydrogen peroxide to ferrous iron, resulting in the creation of potentially damaging hydroxyl radicals (70). Organisms have highly conserved mechanisms of preventing this reaction in a harmful context, primarily through the binding of ferric iron to ferritin and transferrin receptor, allowing for safe storage and distribution of iron. However, although this reaction is of concern in the setting of elevated iron levels, there is extensive evidence demonstrating the role of iron in antioxidant contexts.

Erythroid cells require the iron-containing prosthetic group heme for α- and β-globin chain synthesis during reticulocyte maturation. Although free heme has cytotoxic effects, it can also act as a signaling molecule, triggering the canonical cytoprotective hemeoxygenase-1 (HO-1) transcriptional program. Heme interacts directly with Bach1, a transcriptional repressor, to deactivate it and allow for transcription of a multitude of genes in the metabolism of heme, including HO-1 (71). An additional mechanism includes heme-mediated stabilization of the transcription factor NF-E2–related factor 2 (Nrf2), a potent regulator of antioxidant proteins for which there is experimental evidence suggesting a protective effect in pulmonary vascular disease (72). This tightly regulated program likely serves as a protective mechanism in periods of high oxidant stress. Oxidative stress is ubiquitous and is increased in many disease states. Iron is a central mediator of this, as illustrated by the hemoglobinopathies.

Hemoglobinopathies and PH

It has been suggested that hemoglobinopathies may be one of the most common causes of PH worldwide owing to the high prevalence of this heterogeneous group of diseases (73). Nearly every form

of hemolytic anemia, including sickle cell disease, thalassemia, hereditary spherocytosis, paroxysmal nocturnal hemoglobinuria, and microangiopathic hemolytic anemias, has been associated with PH (74). Homozygous sickle cell anemia is frequently associated with PH, with several studies demonstrating prevalence by echocardiographic criteria at 30% (75).

Although the pathobiology of these hemolytic disorders is heterogeneous, there may be a shared pathophysiology that gives rise to PH. Several experimental and clinical studies have demonstrated that chronic hemolysis results in nitric oxide depletion, increased endothelin-1, and augmented platelet activation, all of which have pathogenic links with PH (76). Iron loading occurs in B-thalassemia major primarily from blood transfusions; however, in non–transfusion-dependent thalassemia, iron overload is still a frequent occurrence, thought to result from ineffective erythropoiesis and hypoxia, culminating in suppression of hepcidin activity, which leads to increased iron uptake (77). In addition to nitric oxide depletion, there may be increased oxidant formation and myocardial iron deposition contributing to associated cardiovascular disease (74, 78). Emphasizing the biologic importance of iron overload, human studies have demonstrated that chelation therapy in thalassemia intermedia results in a significant reduction in pulmonary arterial pressures by echocardiogram (79).

Given the significant global health burden of hemoglobinopathy-associated PH and the lack of effective therapies (75), further research is necessary to delineate the mechanisms by which these disorders lead to pulmonary vascular disease, which could inform unique iron-targeted therapeutics.

Iron, Metabolic Dysregulation, and Mitochondrial Dysfunction

Many pathologic and molecular features of PH, including apoptotic resistance, increased cellular proliferation, and a preferential aerobic glycolysis, are analogous with characteristics seen in cancers (80, 81). These are supported by complementary human and experimental studies. Further contributing to the quasi-malignancy paradigm of PH is evidence of clonal endothelial cell expansion in patients with IPAH (82), somatic chromosomal abnormalities in IPAH lungs (83), increased uptake of 18-fluorodeoxy-glucose in the lung fields of patients with IPAH (84), and the presence of DNA microsatellite instability within the IPAH vascular proliferative lesions (85). The metabolic switch toward aerobic glycolysis and mitochondrial dysfunction in PH is increasingly recognized as a key pathophysiologic feature shared with cancers.

Experimentally, rodents with PH exhibit aerobic glycolysis- and HIF-1 α–dependent vascular cell growth and PH (86); this is paralleled by increased uptake of 18-fluorodeoxy-glucose uptake, as observed in humans with the disease (56). HIF activity may be intimately tied to this pathologic shift because hormone response element promoter transcription results in target gene expression, resulting in a shift from oxidative phosphorylation to glycolysis (87). Highlighting the wide reach of HIF-regulated cellular control, microRNA-210 has been identified as a transcriptional target of HIF (88) and has been implicated in suppression of the ISC assembly proteins, effectively down-regulating mitochondrial respiratory complexes via decreasing ISC biogenesis (89). As detailed above, deregulated iron metabolism has the ability to either up-regulate or attenuate HIF, providing a potential pathobiologic link in PH.

Mitochondria are the synthetic site of ISCs and heme, which function as prosthetic groups that are essential for numerous cellular functions. Heme in addition to its critical role in erythroid cells, has antioxidant effects. ISCs are integrated into proteins within the electron transport chain and into enzymes within the Krebs cycle, such as aconitase and succinate dehydrogenase (90). Although it remains unclear whether altered iron availability contributes to the pathobiology of PH through disruption of this metabolic machinery, there is evidence that mutations in NFU1—a protein that participates in iron sulfur complex assembly—results in a fatal mitochondrial disease, with PH being a feature in 70% of the individuals identified (91).

Given the fundamental role of iron in metabolism and mitochondrial function, there are several potential mechanisms by which altered iron availability may contribute to the pathobiology of PH. Future work will need to determine the importance of ISC availability on mitochondrial function and pulmonary vascular cell metabolism as well as the role of HIF in this relationship.

Conclusion

Iron deficiency has a striking prevalence and association with increased morbidity and mortality in various forms of PH. It is

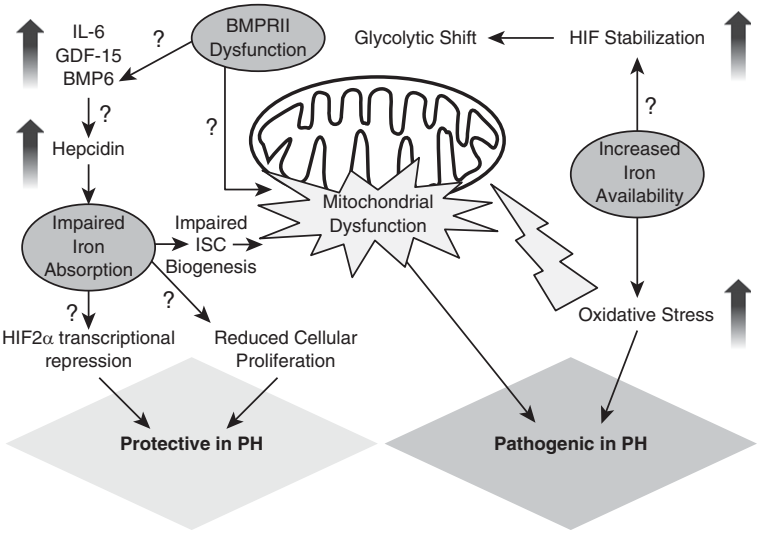

Figure 3. Proposed mechanisms of dysregulated iron metabolism and relation to the pathobiology of pulmonary hypertension (PH). Iron availability has the potential to positively or negatively affect key pathobiologic features of PH depending on the circumstance. Hypothetical and unclear relationships are indicated by a question mark. BMP6, bone morphogenic protein 6; BMPRII, bone morphogenic protein receptor II; GDF-15, growth differentiation factor 15.

a compelling therapeutic target given the relative ease with which it can be manipulated via supplementation and chelators. However, iron has such critical and wide-ranging cellular roles (including HIF regulation, cellular metabolism, and oxidative stress) that specific mechanisms must first be delineated given the potential pathogenic role of either increased or decreased iron availability (summarized in Figure 3). It remains to be shown whether systemic iron deficiency results in tissue- or cell-specific iron depletion within the pulmonary vasculature. Parsing the mechanisms of iron on cellular function is of critical importance to understanding the role of iron in the pathobiology of PH. Furthermore, with iron deficiency affecting an estimated 2 billion people worldwide (92), a deeper understanding of how iron availability alters such fundamental cellular processes has broad implications for human disease. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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